

FEB 23 2006

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Applicants: Edith Mathiowitz, Yong Shik Jong and Kim Boekelheide

Serial No.: 10/663,265

Art Unit:

1632

Filed: September 16, 2003

Examiner:

Leavitt

For: POLYMERIC GENE DELIVERY SYSTEM

Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450**DECLARATION UNDER 37 C.F.R. 1.131**

Sir,

We, Edith Mathiowitz, Yong Shik Jong and Kim Boekelheide, hereby declare:

1. We are the inventors of the subject matter claimed in the above-identified patent application.
2. We conceived and reduced to practice the subject matter of the claims in the above-identified patent application at least as early as February 22, 1993.
3. This is demonstrated by the attached summary of experiments, including methods, materials and results, conducted prior to February 22, 1993, which was provided to the Technology Licensing Office on April 15, 1994. The dates have been removed.

Applicants: Edith Mathiowitz, Yong Shik Jong and Kim Boekelheide  
Serial No.: 10/663,265  
DECLARATION UNDER 37 C.F.R. 1.131

4. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further, that these statements are made with the knowledge that willful false statements are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: Feb. 21, 2006

Edith Mathiowitz  
Edith Mathiowitz

Date: 2.21.2006

Yong Shik Jong  
Yong Shik Jong

Date: \_\_\_\_\_

Kim Boekelheide  
Kim Boekelheide

Serial No.: 10/663,265

## DECLARATION UNDER 37 C.F.R. 1.131

4. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further, that these statements are made with the knowledge that willful false statements are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: \_\_\_\_\_

Edith Mathiowitz

Date: \_\_\_\_\_

Yong Shik Jong

Date: 2/22/06  
Kim Böckelheide

## Gene Therapy Project Summary

## Preliminary Study

1 Encapsulation of  $\beta$ -gal coding DNA linear and supercoiled in a PLA blend

- Materials: 1 g PLA (300K) dissolved in 5 ml of methylene chloride

2 g PLA (2K) dissolved in 5 ml of methylene chloride

$\beta$ -gal plasmid (1-2 mg/ml), diluted 1: 5

- Methods: The two solutions were mixed (no phase separation observed).

5 drops of Span 85 was mixed into this solution.

The resulting mixture was aliquoted into glass vials (2 ml/vial).

In each glass vial, 100  $\mu$ l of DNA (20  $\mu$ g-40 $\mu$ g) was added.

The glass vials were left in the refrigerator for two days and lyophilized.

- Note: After addition of the DNA solution, the polymer blend precipitated quickly and droplets of DNA were visible under optical microscopy.

Implantation of DNA/PLA pellets

- Sterilization: EtOH 5 min  
PBS-P/S 5 min

- Surgery: Each rat received linear DNA into the left leg and supercoiled DNA into the right. Implants were inserted into incised muscle - either the vastus or the hamstring. The muscle was sutured back together and then the skin.

<u>Rat ID</u>	<u>Implant Duration</u>	
R1	11/6 - 11/20/91	2 weeks
R2	11/6 - 3/6/92	4 months
R116	8/19 - 9/8/93	3 weeks

- **Explant:** Rats were perfused with PBS/heparin followed by 3% paraformaldehyde and 0.2% glutaraldehyde in PBS. Post-fix with 3% paraformaldehyde followed by 15% sucrose/PBS. Excised muscles were cut with a cryostat and stained with X-Gal.